

3. INTRODUCTION

3.1 INVESTIGATIONAL PROPOSAL

Leuvectin is an immunotherapeutic agent containing a DNA plasmid that encodes the IL-2 protein. Vical Inc. is actively investigating Leuvectin for treatment of several types of cancer. This protocol is intended to provide a controlled means for providing retreatment with Leuvectin to former clinical study patients who continue to show a clinical benefit after Leuvectin study completion. A patient considered to be a candidate for retreatment under this protocol could receive additional courses of Leuvectin immunotherapy as long as they continue to satisfy enrollment criteria, the investigator deems continued treatment to be in the patient's best interest, and Vical continues to be both willing and able to provide the study material.

3.2 OVERVIEW

Immunotherapy has shown promise as an approach to the treatment of malignancy. The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells. This may be achieved by modifying either the tumor cells or the host response causing various lymphocyte populations, particularly cytotoxic T lymphocytes (CTLs), to respond specifically to tumor cell antigens. Cancers such as melanoma and renal cell carcinoma are sometimes responsive to immunotherapy because the immune system can be induced to recognize tumor-associated and tumor-specific antigens in these cells.

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells by mobilization of either cellular or humoral immune effectors. Cellular mediators of antitumor activity include MHC-restricted cytotoxic T cells (CTLs), natural killer (NK) cells (1,2) and lymphokine-activated killer (LAK) cells (3). CTLs which infiltrate tumors have been isolated and characterized (4). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumors from which they have been derived (5,6). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (7,8), or by activation induced by substances such as Bacillus Calmette-Guerin (BCG) (9).

Cytokines also participate in the antitumor response by direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor- α (TNF- α), interferon- α (IFN- α), interferon- γ (IFN- γ) and lymphotoxin can result in neoplastic cell death (10,11). Interferon- γ markedly increases class I and II MHC cell surface expression (12,13) and synergizes with TNF- α in producing this effect (14). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (15), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine

activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (3, 16, 17). The LAK cells lyse tumor cells without preimmunization or MHC restriction (18). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor-specific killer cells (19).

Systemic administration of IL-2 alone, or IL-2 with LAK cells has been shown to upregulate the immune system resulting in tumor regression; however, significant side effects result as well (20). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells.

3.3 LEUVECTIN

Scientists at Vical Inc. have developed a direct gene transfer method to transfect tumor cells with genes encoding immunomodulating proteins. The Vical approach introduces the recombinant gene encoding the IL-2 protein directly into malignant tumor cells *in vivo* which eliminates the need to establish cell lines from each patient and minimizes delays in the time to treatment. Additionally, no viral vectors are contained in the formulation. The product, Leuvectin, is composed of plasmid DNA coding for IL-2 (VCL-1102) formulated in an injection vehicle with DMRIE/DOPE, a proprietary cationic lipid mixture (cytofectin). When introduced into the target tumor, the lipid facilitates transfection of the tumor cells. The IL-2 gene product is expressed and secreted at the tumor site.

In developing Leuvectin, Vical conducted pharmacology and toxicology studies to: 1) demonstrate that the plasmid/lipid complex results in transfection and the plasmid produces biologically active IL-2 protein in tumors, 2) demonstrate that VCL-1102 reduces tumor burden in a mouse tumor model, and 3) explore the effect of injecting the plasmid directly into normal mouse liver. Results of the studies showed that: 1) the IL-2 plasmid/lipid formulation produced a high level of IL-2 protein expression in injected tumors, 2) the direct intratumoral injection of a plasmid DNA expression vector encoding the human IL-2 gene into subcutaneous B16 melanoma or renal cell carcinoma tumors in mice significantly slowed tumor growth and reduced the incidence of palpable tumors, and 3) intrahepatic administration of Leuvectin in mice was well tolerated and there were no adverse effects associated with the drug; adverse effects that occurred were attributed to the injection procedure and not considered to be of biological significance. Details of these pharmacology and toxicology studies are contained in the Investigator's Brochure.

Animal drug safety studies were conducted to: 1) assess the acute toxicity of Leuvectin in mice at multiples of the highest anticipated human dose, 2) evaluate

the effect of repeat administration in mice over an extended period, and 3) evaluate the effect of repeat-dose administration in Cynomolgous monkeys. No acute or residual toxic effects were observed in these animal safety studies. Details of these studies are contained in the Investigator's Brochure.